# ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

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# ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

A manuscript entitled "The Influence of Spore Moisture Content on the Dry Heat Resistance of Bacillus subtilis var. niger" has been prepared. This manuscript describes the techniques developed through Research Project R-36-015-001 for measuring the dry heat resistance of B. subtilis var. niger spores located in and on various materials. D and z values are presented for these spores in the various test systems and the manuscript includes data collected during the Eleventh Quarter (October 1 - December 31, 1967).

An explanation of the differences in heat resistance as related to the materials in or on which spores are located is offered and is concerned with spore moisture content or water activity. The manuscript serves as a summarization of the findings so far observed on this project and may be viewed as a current status report. With this in mind, the above titled manuscript is offered as the Eleventh Quarterly Report of Progress and is attached.

The Twelfth Quarterly Report of Progress (January 1 - March 31, 1968) will present data on thermal resistance as affected by various rates of spore hydration and dehydration resulting from storage of spores at different relative humidities. In addition, data will be presented on the effects of spore moisture content on $z_D$ values.

# THE INFLUENCE OF SPORE MOISTURE CONTENT ON THE DRY HEAT RESISTANCE OF *BACILLUS SUBTILIS* VAR. *NIGER*

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The dry heat resistance of *Bacillus subtilis* var. *niger* spores located in or on various materials was determined as D and z values in the range of 105 through 160 C. The systems tested included spores located on steel and paper strips, spores located between stainless steel washers mated together under 150 in.-lbs. and 12 in.-lbs. of torque and spores encapsulated in methylmethacrylate and epoxy plastics. D values for a given temperature varied with the test system. High D values were observed for the systems in which spores were encapsulated or under heavy torque, whereas, lower D values were observed for the steel and paper strip systems and the lightly torqued system. Similar z values were obtained for the plastic and steel strip systems ($z_D$ = 21 C) but an unusually low z for spores on paper ($z_D$ = 12.9 C) and an unusually high z for spores on steel washers mated at 150 in.-lbs. of torque ($z_D$ = 32 C) were observed. The effect of spore moisture content on the D value of spores encapsulated in water impermeable plastic was determined and maximum resistance was observed for spores with a water activity ($a_w$) of 0.2 to 0.4. Significantly decreased D values were observed for spores with moisture contents below

$a_w$ 0.2 or above $a_w$ 0.4. The data indicate that important factors to be considered when measuring the dry heat resistance of spores are a) the initial moisture content of the spore; b) the rate of spore desiccation during heating; c) the water retaining capacity of the material in or on which the spores are located; and d) the relative humidity of the system at the test temperature.

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The dry heat sterilization cycles presently employed have been empirically derived (14,22) and are too severe to apply to the sterilization of interplanetary spacecraft. Because of the microbial contamination associated with the interiors of certain electronic parts (15), the National Aeronautics and Space Administration has decided that interplanetary spacecraft shall be sterilized. Dry heat has been assessed as one of the sterilization processes that may be applied to spacecraft provided that time-temperature combinations can be developed that are compatible with maintaining the functional properties of electronic parts.

In comparison to the accumulated knowledge of the factors that influence microbial resistance to wet heat and except for the reports of Murrell and Scott (8,12) on the effects of water activity, little is known of the factors that affect microbial resistance to dry heat. In an attempt to identify some of these factors and to generate data that may be useful in developing spacecraft sterilization cycles, this study has been undertaken. This report describes the dry heat resistance of microbial spores in the range of 105 to 160 C as influenced by initial spore moisture content

and by systems which because of their physical characteristics either permit or retard spore desiccation during heating.

## MATERIALS AND METHODS

Production of spores. Bacillus subtilis var. niger spores were produced by surface culture on agar medium (Seitz filtered glucose, 0.25%; Casamino acids (tech.), 0.25%; yeast extract, 0.5%; $MnSO_4.H_2O$, 0.001%; $FeSO_4.7H_2O$, 0.0014%; agar, 3%) in six liter Pavitsky bottles with 7 days incubation at 35 C. The spores were washed from the surface with double distilled sterile water, shaken with glass beads, filtered through cotton and held at 45 C (waterbath) overnight. The heated suspension was washed 5 times in 400 ml volumes of double distilled sterile water (4,080 x G for 20 min at 5 C) and stored at 5 C. Sufficient spore crops were produced initially so that a single, pooled, stock spore suspension could be used throughout the course of the study. Plate count values of the refrigerated stock spore suspension prepared in tryptone glucose beef extract agar (48 hr incubation at 35 C) obtained at various intervals over the past 2-1/2 years has revealed no change, with time, in the number of spores per ml. Additionally, no difference in the total number of spores per ml has been observed over the same period for heat-shocked (80 C for 10 min) versus non-heat shocked aliquots of the stock spore suspension.

Steel and paper test surfaces. By means of a microburette 0.01 ml aliquots of an appropriate aqueous dilution of the stock spore suspension were placed on one surface of 1 x 1/4 inch strips of stainless steel (202, #4 finish) and filter paper (Whatman #2). The strips were placed in 13 x 100 mm

borosilicate thermal death time (TDT) tubes which were then constricted to form a well in the upper third of the tube. A loose plug of glass wool was placed in the constriction and the well was filled with silica gel and loosely plugged with cotton (See Fig. 1). The tubes were placed in a forced air oven at 50 C for 1 hr, after which they were placed in a desiccator over silica gel and held overnight at 20 C. Following drying, the tubes were sealed at the constriction with an oxy-gas torch. The silica gel in the well above the constriction prevented the reintroduction of moisture from the ambient air during sealing.

Stainless steel washers (202, #4 finish, 1/2" O.D. and 1/4" I.D.) also were inoculated on one surface with 0.01 ml of the aqueous spore suspension and dried at 50 C for 1 hr as described above. The dry, inoculated washers were mounted on the male lug (See Fig. 2) with the inoculated surface adjacent to the uninoculated surface of a second washer. Male and female lugs were loosely threaded together, placed in 15 x 100 mm borosilicate TDT tubes, and the tubes and torque wrench were sealed in a polyethylene bag containing silica gel. The flexible desiccator was stored over night at 20 C. Working from outside of the plastic bag, the units were torqued the desired amount, the bag was opened, and a constriction was made in the TDT tubes. Silica gel was placed in the upper well of the tubes, and the tubes were stored in a desiccator over silica gel overnight at 20 C, after which the tubes were sealed at the constriction. Again, these precautions were taken to assure that moisture from the ambient air did not contact the dried spores located on the mated surfaces.

Fabrication of lucite (methylmethacrylate) rods. To remove the polymerization inhibitor, methylmethacrylate monomer was washed twice with equal volumes of 2% NaOH, followed by two additional washings with equal volumes of distilled water. The washed monomer was then mixed with an excess of anhydrous sodium sulfate ($Na_2SO_4$) and allowed to stand overnight to remove water. The sodium sulfate was removed by filtration and the monomer was stored in the cold (5 C) until ready for use.

One ml of an aqueous dilution of the stock spore suspension was distributed over 50 grams of methylmethacrylate powder contained in a sterile drying pan. The pan was placed in a forced air drying oven for 30 minutes at 50 C, removed and the powder placed in a sterile motar and ground by hand until the dried spore inoculum appeared to be evenly distributed throughout the powder. The powder was returned to the drying pan, heated an additional 30 minutes in the oven, and once more ground. The desired quantity of inoculated, dried powder was weighed into a shallow pan and placed in a desiccator over silica gel and held overnight at 20 C. Following overnight storage, the powder was transferred to a beaker and to each 50 grams of powder, 50 ml of methylmethacrylate monomer was added and mixed with a spatula. Though some moisture may have contacted the inoculated powder during the transfer to the beaker, it was assumed that after the addition of the monomer absorption of moisture would be negligible because of the relative water impermeability of the finished plastic. The liquid mixture was placed in a vacuum flask and evacuated with a water pump until bubbles no longer formed. The viscous, partially polymerized plastic was poured into TDT tubes and the tubes were cotton stoppered and placed in a

50 C water bath for 2 hr to complete the polymerization. Following polymerization, the TDT tubes were sealed in the oxy-gas flame. This method consistently yielded lucite rods that were hard, clear, free of bubbles, and from which approximately 1 x $10^8$ spores per gram were consistently recovered (See Recovery Methods). An analysis for the distribution of spores in the rods revealed that no significant differences ($\alpha$ = 0.05) in spore concentrations were detected within a rod or among rods prepared from separate batches of plastic and that the viable spore population within the rods remained stable when the rods were stored for several days at room temperature.

Fabrication of epoxy rods. Solid blocks of epoxy plastic were formed by mixing Scotchcast Electrical Resin No. 5 (Minnesota Mining and Manufacturing) Part A (syrup) with Part B (hardner) in a 2:1 ratio in a beaker that had been previously coated with Mold Release 225 (Ram Chemicals, Inc.). The release agent was applied to the beaker with a cotton swab and the coated beaker was baked at 107.2 C (225 F) for 1 hr. The beaker of casting syrup was placed in a 50 C water bath for 3 hr during which time the plastic polymerized. The epoxy block was disintegrated by means of an electrical sanding belt and the shavings were collected, autoclaved for 15 min at 121 C, and dried overnight in the 50 C forced air oven. Sixteen grams of shavings, in a drying pan, were inoculated with 6.4 ml of the undiluted, aqueous stock spore suspension. The inoculum was dried and ground twice as described for methylmethacrylate and stored over silica gel overnight at 20 C. To each 16 grams of inoculated and desiccated

shavings, 64 grams of Scotchcast Resin Part A was added, mixed thoroughly, and followed by the addition of 48 grams of Part B. Mixing, with a spatula, was continued until the whole appeared homogenous. The syrup was evacuated similarly to methylmethacrylate, poured into cotton stoppered TDT tubes that had been previously coated with release agent (see above) and placed in the 50 C water bath for 3 hr to polymerize. Following polymerization, the tubes were sealed in the oxy-gas flame. This method consistently yielded rods that were rigid, clear, free of bubbles, and from which approximately 1 x $10^8$ spores per gram were recovered consistently (See Recovery Methods).

Equilibration of spore moisture content. Water activity ($a_w$) is a property of aqueous solutions and is defined as $a_w = p/p_o$, where p and $p_o$ are the vapor pressure of the solution and solvent, respectively. The connection between relative humidity and water activity is that $a_w$ is numerically equal to the relative humidity expressed as the fraction RH/100. Under conditions of water vapor equilibrium or equilibrium relative humidity, the terms $a_w$, RH, and ERH are interchangeable and define each other. For a detailed discussion of the $a_w$ concept, see Scott (20,21).

To determine the effect of spore moisture content on resistance, methylmethacrylate powder was inoculated and dried as described above. The desired quantity (50 g) of dried and inoculated powder was placed in a pan and spread out to achieve a shallow layer of powder. The pan was placed in a desiccator containing 500 ml of a saturated salt solution. The desired saturated salt solution required to yield a vapor pressure of known water activity ($a_w$) or equilibrium relative humidity (ERH) at

25 C in a sealed container was selected from Robinson and Stokes (18). The desiccator was sealed and stored for 14 days at 25 C ± 0.5 C after which 50 ml of methylmethacrylate monomer was added to the equilibrated powder immediately upon removal of the desiccator lid. Lucite rods were formed from this material and sealed in TDT tubes as previously described.

Determination of dry-heat resistance. Replicate TDT tubes containing the various inoculated test materials were heated by complete immersion in a silicone bath operating at the desired test temperatures (± 0.1 C). Following the heat exposure, the TDT tubes were plunged immediately into ice-water to cool for 15 min followed by washing in detergent solution to remove the silicone. After washing and rinsing, the tubes were immersed in saturated alcoholic iodine solution for 10 min, dried with sterile towels, scored, and snapped open. The contents were removed aseptically and examined bacteriologically as described under Recovery Methods. Heat penetration curves were obtained for each test material and corrections for lethality during "heat-up" and "cool-down" were calculated by the graphical method (1). These corrections were applied whenever the combined "heat-up" and "cool-down" time was equivalent to or exceeded three per cent of the total experimental time exposure.

Calculation of D and z values. Identical experiments (I and II) were duplicated for each test temperature. Paired samples (a and b) were tested at each exposure time within an experiment. Plate counts, in duplicate, ($a_1$ and $a_2$;$b_1$ and $b_2$), were obtained for each of the paired samples. This resulted in the use of four observations per time interval for each of the

paired samples in a given experiment. Linear regressions were calculated for the data from each experiment. The regression so obtained was tested for homogeneity of regression to the data obtained for the second experiment at the same temperature. At times, a nonhomogeneity of regression was observed for the data from duplicated experiments. In these instances, the experiments were repeated with special care to observe good laboratory technique and nonhomogeneity of regression was eliminated. The proportion of the sum of squares of deviations ($R^2$) due to linear regression were calculated as well as the D value (the time interval at the test temperature required to obtain a 90% reduction in the number of viable spores) and the 95% confidence interval for the D value. To obtain $z_D$ values (slope of the thermal destruction curve or the number of degrees required for the thermal destruction curve to traverse one log cycle) linear regressions were calculated of the D values so derived. The general method and assumptions for fitting linear regression, testing of homogeneity, and calculating confidence intervals were that of Ostle (13).

Recovery Methods. Paper strips were placed in 100 ml of sterile phosphate buffered dilution water (17) contained in a micro-Waring Blendor cup and were blended for two minutes at slow speed. Additional ten-fold serial dilutions were prepared in the same buffer and plated in tryptone, glucose, beef extract agar. The plates were counted after incubation for 48 hr at 35 C.

Steel strips and washers were examined by placing the individual strips or washers in separate 15 x 150 mm test tubes containing ether 5 ml (washers) or 6 ml (strips) of phosphate buffered dilution water. The tubes

were placed in an ultrasonic bath (power output of 300 watts at a frequency of 25 KC/sec) and treated for 12 minutes. A dilution plate count of the liquid contents of the tubes was made as described for paper.

Lucite rods were removed aseptically from the TDT tubes, placed in a sterile screw capped tube and weighed. After weighing, the rod was aseptically introduced through an entry port to a modified Waring Blendor cup containing 200 ml of sterile acetone (See Fig.3). The blendor modification consisted of a stainless steel disc that had been cut from a vegetable grater and mounted in place of the rotary knives. The closure for the jar consisted of a threaded lid fitted with a piece of 1/2 inch diameter stainless steel tubing (the entry port) that projected through the lid. The exposed end of the steel tubing was fitted with a Morton closure and the open end, inside the blendor cup, was positioned approximately 3/16 of an inch above the grinding disc (See Fig.4). The plastic rod was dropped down the steel tubing and a sterile steel rod was inserted that acted as a piston to drive the rod against the grinding disc (See Fig.5). Approximately 1/2 inch of plastic was ground from the rod and the remainder of the rod was withdrawn from the tubing by impaling it with an elongated sterile bodkin. The entry port was again sealed with the Morton closure and the blendor was placed upon a reciprocating shaker (144 strokes per min.) for 1/2 hour at room temperature during which time the plastic shavings were completely dissolved. Serial ten-fold dilutions of the blendor contents were prepared in sterile acetone and one ml or more, as appropriate, of the dilution was passed through a Gelman alpha 6 metricel membrane filter followed by two 10 ml rinses with sterile acetone. The membrane was

placed in a sterile Petri dish to dry. Drying was completed usually within 30 seconds, after which the membrane was overlaid with approximately 20 ml of sterile tryptone, glucose beef extract agar. The membranes were incubated for 18 to 24 hours at 35 C and colonies counted (See Tables 1 and 2 for the effect of acetone on the outgrowth and colonial development of heat treated spores). The weight of the plastic rod was again obtained after grinding and the number of surviving spores per gram of plastic was calculated.

Epoxy rods also were placed in tubes and weighed. After weighing, the rod was inserted into the entry port of the modified Waring Blendor described above. In this case, however, the stainless steel grinding disc used to disintegrate methylmethacrylate rods was replaced with a disc of 220 A grit silicon carbide grinding paper that had been previously leached to remove soluble toxic residues (100 discs autoclaved in 5 liters of distilled water, followed by distilled water rinsing and air drying). The blendor cup contained 200 ml of sterile tryptone, glucose beef extract broth with 0.004% Dow Corning antifoam AF added. A one-half inch section of the rod was ground by applying a 2000 gram weight to the piston. Tenfold serial dilutions were prepared of the ground suspension in phosphate buffered water and were plated in tryptone, glucose beef extract agar made up to contain 5 ml of Tween 80 and 0.7 gram of Asolectin per liter (phenol neutralizer). The plates were incubated 72 hours at 35 C and counted.

## RESULTS

Survivor curves of B. subtilis var. niger spores were plotted from the data obtained in each heating experiment. With the exception of the lucite system, a straight line, logarithmic order of death was observed for all the systems. A typical curve is presented in Figure 6 (epoxy 115 C) in which the linear regression of the survival points is represented by the solid line and the 95% confidence interval for the linear regression is presented as the dashed lines. The lucite curves typically were diphasic and a sharp decrease in numbers (approximately 99%) was noted during the initial heating period followed by a slower rate of death for the remainder of the exposure. The $R^2$ value for the lucite experiments often was .90 or more despite the initial die-off and D values were calculated similarly to those for the other systems.

The D values for the test spores obtained with the various systems are presented in Table 3. Spore resistance to a given dry heat temperature varied with the carrier in or on which the spores were located. Spores encapsulated in plastics or trapped between stainless steel surfaces under heavy torque (150 inch-pounds) displayed greater resistance than those located on paper and on stainless steel strips or located between steel surfaces under light torque (12 inch-pounds). For example, at 125 C, an approximate 38-fold increase in resistance was noted for spores encapsulated in epoxy versus that observed for spores on stainless steel strips. The relative resistance of the spores at 135 C for the various test systems is presented graphically in Figure 7.

Thermal destruction (TD) curves and their corresponding $z_D$ values were calculated from linear regressions of the semilogarithmic plot of D values and their corresponding temperatures. The values so obtained along with their 95% confidence intervals are presented in Table 4, whereas, the curves derived from these plots are depicted in Figure 8. On conversion to the Fahrenheit scale and with the exception of the paper data, these values are large (38 to 58 F) in comparison to the $z_D$ value of 16 to 20 F in the temperature range of 220 to 270 F associated with spores subjected to wet heat systems (19). By way of contrast, the value for the paper system ($z_D$ = 12.9 C or 23.2 F) approximates that of a wet heat system.

The influence of water activity upon the dry heat resistance of spores encapsulated in lucite is shown in Figure 9. These data indicate that spores of intermediate moisture content are more resistant than spores of lesser or greater moisture content. Maximum resistance occurred in the range of 0.2 to 0.4 $a_w$ and an approximate 8-fold increase in resistance was noted between minimally resistant (0.9 $a_w$, $D_{135}$ = 10.9) and maximally resistant (0.4 $a_w$, $D_{135}$ = 88.7) spores.

## DISCUSSION

Before proceeding with a discussion of the results outlined above, it may be well to review momentarily the definition of dry heat and some of the recent findings pertaining to spore permeability and water activity.

The term "dry heat" obviously implies the application of heat in the absence of water. On closer examination, however, one becomes aware that some finite value must be established to define the term "dry" or rather its antithesis "wet". A wet- or moist-heat sterilization cycle may be

defined as one in which the organism is in contact with an environment with a water activity of 1.0 or a water-saturated atmosphere. These conditions are met only when the organism is heated in contact with pure water or saturated steam. This definition of wet heat implies that dry heat is not an equally specific condition, but rather a range of conditions that includes such factors as the moisture content of the microorganisms prior to and during heating, the water vapor pressure and flow rate of the gaseous atmosphere in contact with the microorganisms, the chemical and physical composition of the material on or in which the spores are located, and the total pressure of the system.

The works of Gerhardt and Black and their associates (2,3,4,5), of Murrell and Scott (9), Murrell (10), Murrell and Warth (11), and of Lewis and co-workers (6) have demonstrated that spores are highly permeable and that a free exchange of water occurs between the spore and its environment. The water activity of spores may be expected, therefore, to change in relation to the water activity of the suspending fluid or with the relative humidity of the atmospheric environment. The ability of spores to come to water vapor equilibrium with their environment is an important consideration in establishing dry heat sterilization cycles as has been demonstrated by Murrell and Scott (8,12) in experiments which revealed that: a) the amount of moisture associated with spores as a result of equilibration to various water activities prior to heating affects dry heat resistance; b) spores of intermediate moisture content (0.2 to 0.4 $a_w$) are more resistant than spores of greater or lesser moisture content; and c) increased D values may be expected when spores of intermediate water activity

are subjected to dry heating conditions that prevent a change in the moisture content of the spores during heating.

The above information is useful in interpreting the results presented earlier. In all the experiments, except those in which the water activity of spores in lucite purposely was varied, an attempt was made to achieve an initially uniform, but unfortunately unknown, moisture content for spores in all the systems tested. Precautions were taken to prevent or at least minimize any gross change in moisture content of the spores or the carrier systems during manipulations. Additionally, previous tests had demonstrated to our satisfaction that the spore carriers were non-toxic and that quantitative recovery of survivors was being achieved. The data of Murrell and Scott (8,12) and those of this study (Fig. 9) reveal that one of the factors which affects dry heat resistance of spores is water activity or the moisture content of the spore prior to and during heating. If the moisture content of the spores and the systems were equivalent at the beginning of each of the heating tests reported herein and the systems were non-toxic, an explanation must be sought for the differences in resistance noted for the various systems (Fig. 7).

Spores that have been dried on surfaces and stored and sealed in TDT tubes in the manner described earlier in this report are in water vapor equilibrium with the gaseous environment within the sealed tube. The equilibrium relative humidity within the tube is temperature dependent and as the temperature increases inside the tube, as a result of immersion in the silicone bath, the moisture bearing capacity of the gas increases and the relative humidity decreases. This results in the diffusion of

of moisture from the spore to the hot gaseous environment. This process continues until the tube contents attain the bath temperature at which time a new water vapor equilibrium is achieved. At this point, the moisture content of the spores and the relative humidity of the system is greatly reduced. To prevent this change in spore moisture content during heating, Murrell and Scott (12) heated spores in sealed tubes containing salt solutions that maintained the same $a_w$ during heating as that to which the spores were initially adjusted. Another method of preventing spore water loss during heating may be accomplished by encapsulating spores in non-permeable plastics. Methylmethacrylate absorbs from 0.3 to 0.4 per cent moisture after immersion in water for 24 hours at room temperature, whereas, epoxy absorbs from 0.05 to 0.1 per cent moisture under the same conditions (16). Because of their impermeability, the diffusion of water from the spores to the plastic, or the reverse, would be negligible.

That little, if any, water loss during heating occurred in spores encapsulated in plastic is made evident by a comparison of the data in Fig. 9 to those of Murrell and Scott (12) which were collected in a system employing a controlling solution that maintained the desired vapor pressure at the test temperature. The shape of the survivor curves observed by both sets of experimentors is similar and both groups observed maximum spore resistance in the range of 0.2 to 0.4 $a_w$. From these data, one may conclude that there appears to be a critical moisture content of spores which provides them maximum protection under dry heating conditions. This moisture content is equivalent to that obtainable by spores when they are equilibrated at relative humidities of 20 to 40% ($a_w$ 0.2 to 0.4) and

according to Marshall et al. (7), this moisture content is in the range of 5.5 to 12.4 per cent of the dry weight of the spores, depending on the species.

The differences in resistance displayed by the spores located in or on the various test materials (Fig.7) may be related to the rate at which the spore moisture content was reduced during heating. Naked systems represented by steel and paper strips would be expected to give up their water to the hot air rapidly, with consequent desiccation of the spores on their surfaces. Water would diffuse from mated surfaces more slowly than from naked surfaces as a function of the mating pressure, and water would diffuse extremely slowly from the plastics. Rapid diffusion of water from the spore to the hot atmosphere within the sealed tube would result in rapid spore desiccation to moisture contents below some critical level (presumably $a_w$ 0.2) at which point rapid destruction rates occur (low D values). Systems which retard or prevent a change in spore moisture content during heating result in slower spore destruction rates (high D values). It appears logical then to attribute the differences in D values shown in Fig. 7 to this mechanism rather than to some unknown character of the material in or on which the spores were located.

A D value is a measure of the rate of death of an organism at a given temperature (time required to obtain a 90% reduction), whereas, a z value is a measure of the change in rate of destruction with temperature and mathematically is equal to the reciprocal of the slope of the thermal destruction curve (TD). The TD curve is constructed by plotting D values logarithmically and temperature arithmetically. Though the D values

observed for each of the systems differed considerably indicating, as previously mentioned, a carrier related effect that may be due to the differences in the water retention capacities of the systems, it is noteworthy that the change in destruction rate with temperature (z) essentially was similar for the plastic and steel strip systems ($z_D$ = 21 C) but quite different for the paper ($z_D$ = 13 C) and mated surface ($z_D$ = 32 C) systems. These differences indicate that the "kill mechanism" may vary among systems. For example, the $z_D$ value for paper indicates a wet heat "kill mechanism", whereas, the high $z_D$ value for mated surfaces is indicative of a mechanism distinct from that observed in any of the other systems. Insufficient information is available presently to explain the differences in observed $z_D$ values. Studies are in progress, however, to establish the relationship between $z_D$ and spore moisture content and to establish whether significant differences existed in the relative humidities within the sealed TDT tubes of the systems studied.

For the present, then, the results reported herein indicate that: a) the initial moisture content of the spores; b) the rate of spore desiccation during heating; and c) the equilibrium relative humidity of the system at temperature influence the dry heat resistance of B. subtilis var. niger spores. The rapidity with which the ERH is achieved within a sealed TDT tube appears to be related to the rate at which water vapor is diffused from the carrier system to the hot atmosphere within the TDT tube. Closed systems (mated systems and water impermeable, plastic encapsulated systems) retard or prevent spore moisture loss during heating, whereas, rapid moisture loss during heating occurs in open systems (naked surfaces).

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TABLE 1. Effect of pre-treatment with acetone on the dry heat inactivation of Bacillus subtilis var. niger spores at 125°C

| Exposure time in hours | Acetone suspension of spores dried on paper | Aqueous suspension of spores dried on paper |
|---|---|---|
| | No. of survivors recovered per strip | No. of survivors recovered per strip |
| 0 | $295 \times 10^6$ | $290 \times 10^6$ |
| 3 | $4.25 \times 10^6$ | $5.05 \times 10^6$ |
| 6 | 750 | $5.7 \times 10^4$ |

TABLE 2. Number of Bacillus subtilis var. niger spores on paper strips surviving one hour exposure to 125°C dry heat in sealed TDT tubes

| Aqueous suspension of spores dried on paper* | | Acetone suspension of spores dried on paper* | |
|---|---|---|---|
| Blended and diluted in acetone | Blended and diluted in water | Blended and diluted in acetone | Blended and diluted in water |
| $13.5 \times 10^7$ | $17 \times 10^7$ | $8 \times 10^7$ | $9 \times 10^7$ |

*Number of spores per strip of paper:

Paper inoculated from water suspension = $20 \times 10^7$ per strip
Paper inoculated from acetone suspension = $15 \times 10^7$ per strip

TABLE 3. D values at various temperatures for Bacillus subtilis var. niger* spores located in or on different materials.

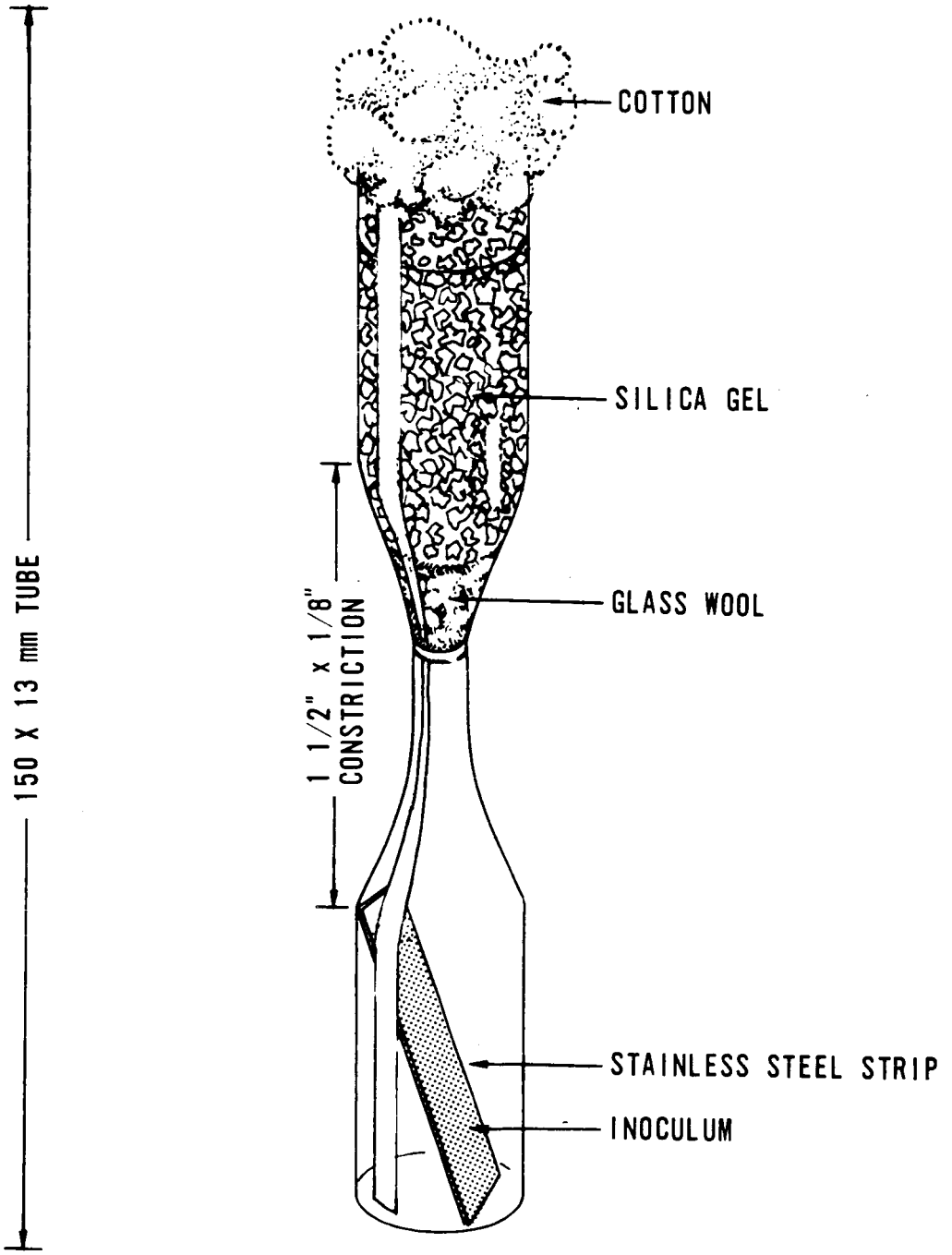
| Dry heat exposure temperature °C | Stainless steel strips | | Paper strips | | Stainless steel washers (150-in.-lbs. torque) | | Lucite rods | | Epoxy rods | |
|---|---|---|---|---|---|---|---|---|---|---|
| | D value | 95% C.I. | D value | 95% C.I. | D value | 95% C.I. | D value | 95% C.I. | D value | 95% C.I. |
| 105 | | | | | | | 32.0 hr.<br>28.8 hr. | 26.4 to 33.6 hr.<br>26.4 to 33.6 hr. | | |
| 115 | 24.0 min.<br>24.4 min. | 21.7 to 27.0 min.<br>22.2 to 27.0 min. | | | 102.6 min.<br>97.8 min. | 95.4 to 111.0 min.<br>90.6 to 106.2 min. | 15.6 hr.<br>15.1 hr. | 13.4 to 17.8 hr.<br>13.7 to 16.2 hr. | 16.1 hr.<br>16.1 hr. | 15.2 to 17.2 hr.<br>15.2 to 16.8 hr. |
| 120 | | | | | | | 6.2 hr.<br>5.9 hr. | 5.6 to 6.7 hr.<br>5.0 to 6.8 hr. | | |
| 125 | 8.3 min.<br>8.9 min. | 7.9 to 8.9 min.<br>8.5 to 9.4 min. | 102.1 min.<br>102.1 min. | 96.1 to 108.2 min.<br>96.1 to 108.2 min. | 47.5 min.<br>44.5 min. | 43.9 to 51.8 min.<br>41.8 to 48.0 min. | 3.1 hr.<br>3.4 hr. | 2.5 to 3.6 hr.<br>2.9 to 3.9 hr. | 5.3 hr.<br>5.3 hr. | 5.1 to 5.7 hr.<br>5.0 to 5.6 hr. |
| 135 | 2.6 min.<br>2.9 min. | 2.6 to 3.0 min.<br>2.3 to 3.8 min. | 16.1 min.<br>17.2 min. | 15.3 to 16.9 min.<br>16.0 to 18.3 min. | 25.5 min.<br>22.0 min. | 20.7 to 33.3 min.<br>18.2 to 27.7 min. | 1.4 hr.<br>1.3 hr. | 1.3 to 1.5 hr.<br>1.2 to 1.4 hr. | 1.9 hr.<br>1.9 hr. | 1.7 to 2.1 hr.<br>1.7 to 2.1 hr. |
| 140 | | | 7.2 min.<br>7.1 min. | 6.8 to 7.6 min.<br>6.6 to 7.5 min. | | | | | | |
| 160 | | | | | | | 4.6 min.<br>4.1 min. | 4.4 to 4.8 min.<br>3.6 to 4.5 min. | | |

* In all tests the initial number of spores equaled 1 x $10^8$ per surface or per gram.

TABLE 4. $Z_D$ values of *Bacillus subtilis* var. *niger* spores obtained in various test systems

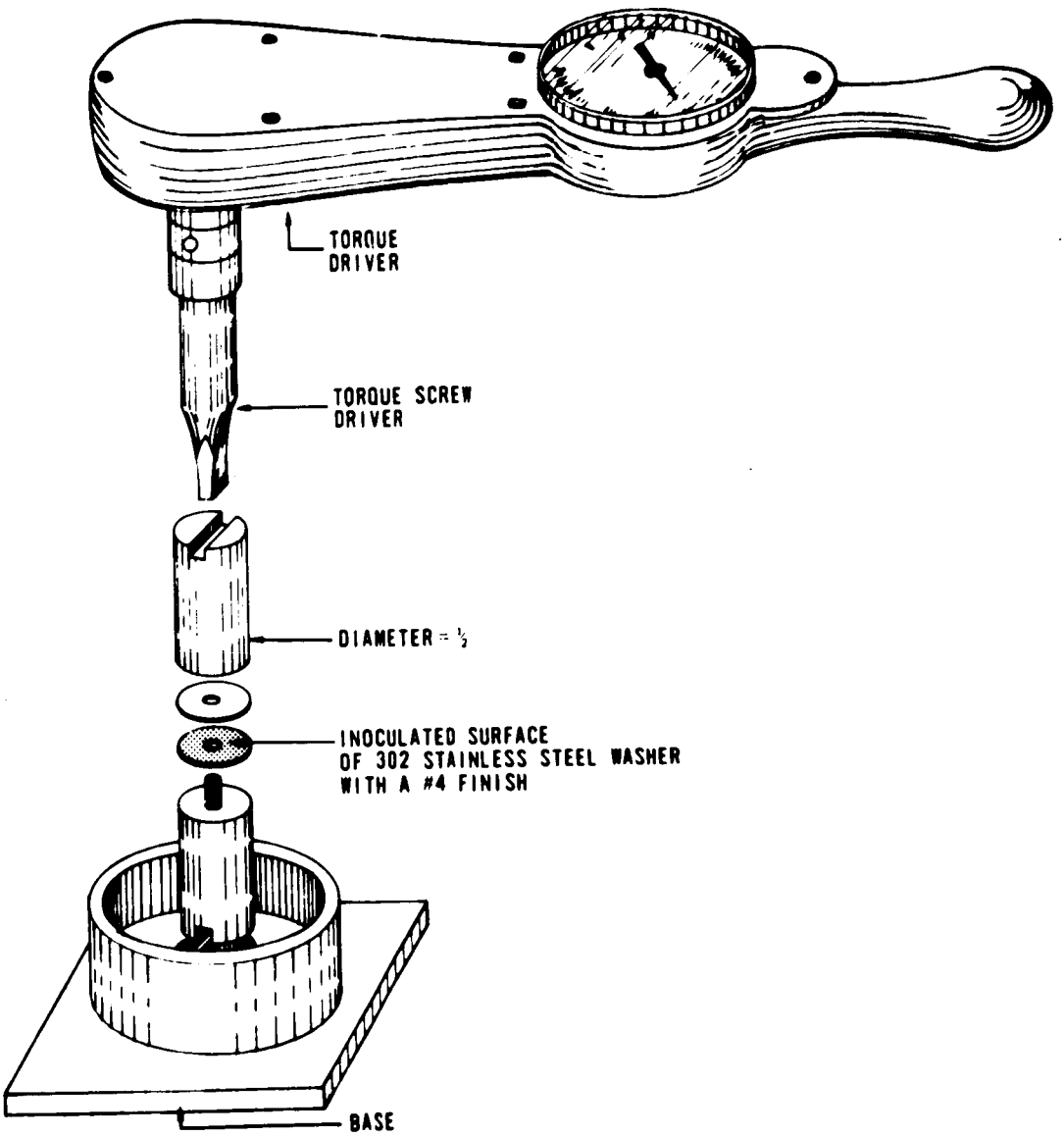
| Test system | $Z_D$ value (C°) | 95% Confidence interval (C°) |
|---|---|---|
| Filter paper | 12.9 | 12.5 to 13.4 |
| Stainless steel (surface) | 20.8 | 19.3 to 22.6 |
| Between stainless steel washers (150 in.-lbs. torque) | 32.0 | 28.2 to 36.8 |
| Lucite (encapsulated) | 20.7 | 19.3 to 22.1 |
| Epoxy (encapsulated) | 21.4 | 20.8 to 22.1 |

FIGURE 1




DRYING TUBE FOR INOCULATED STAINLESS STEEL STRIPS

FIGURE 2




MATED SURFACE ASSEMBLY
(150 in.-lbs.)

FIGURE 3

FIGURE 4

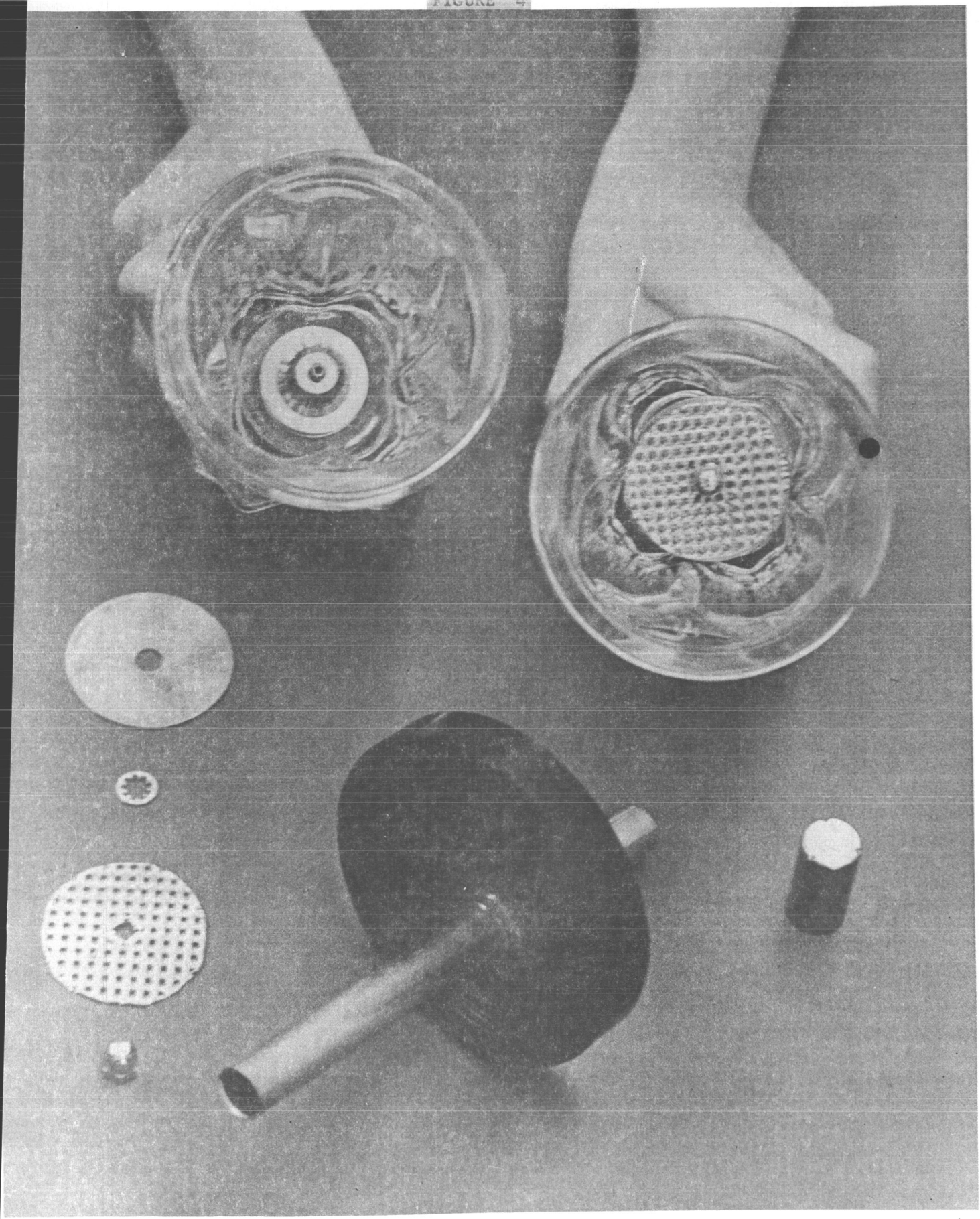
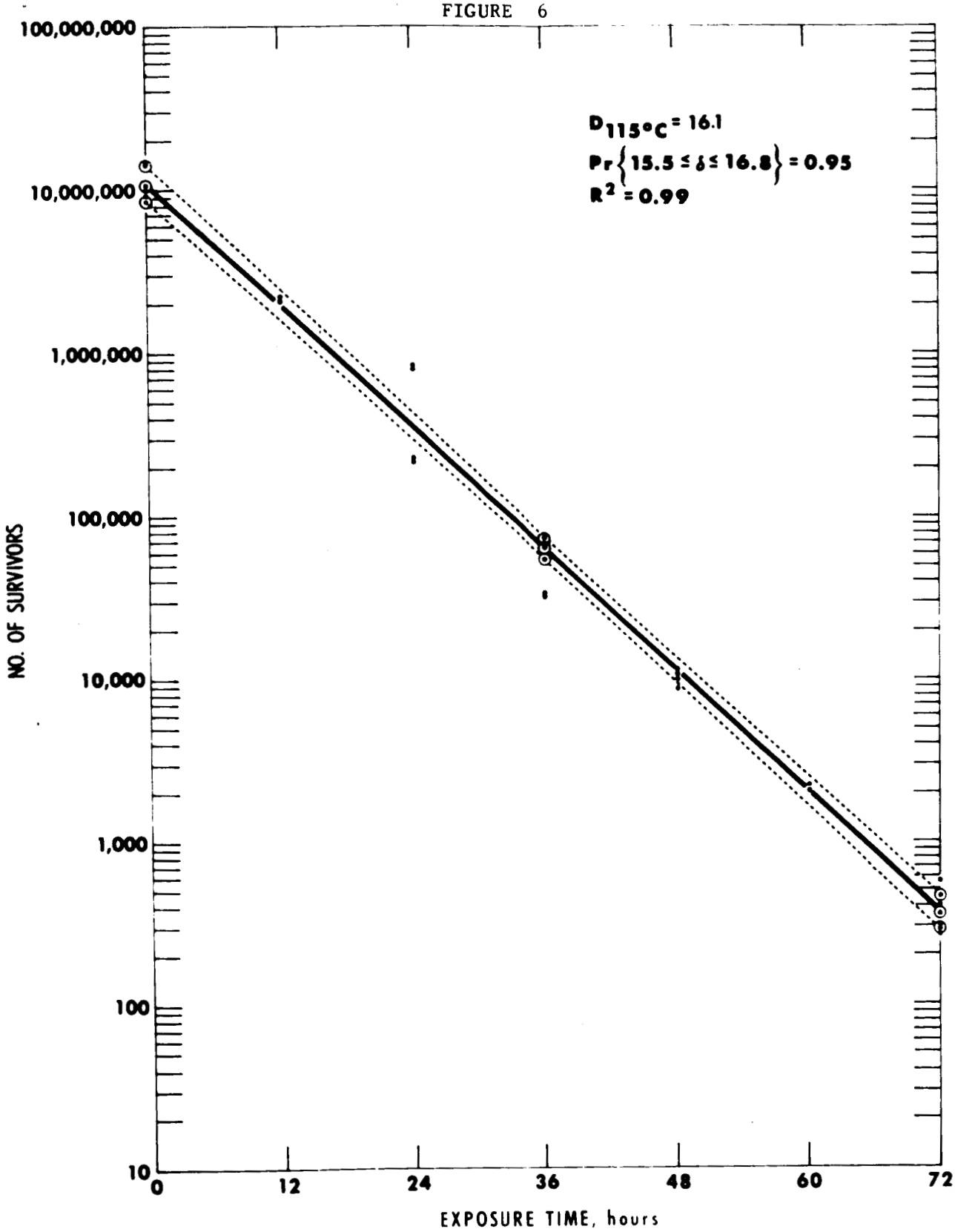


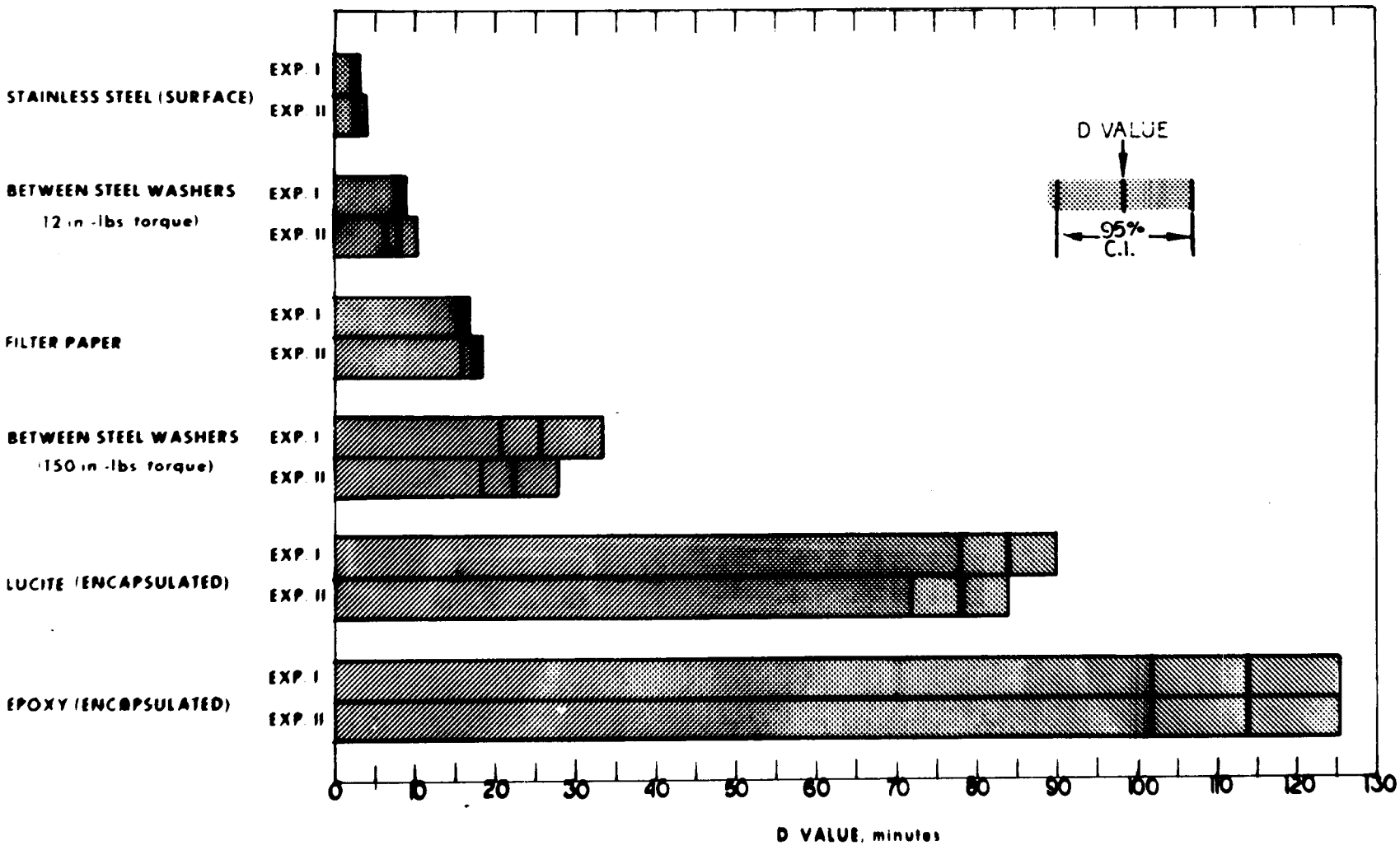
FIGURE 5

FIGURE 6



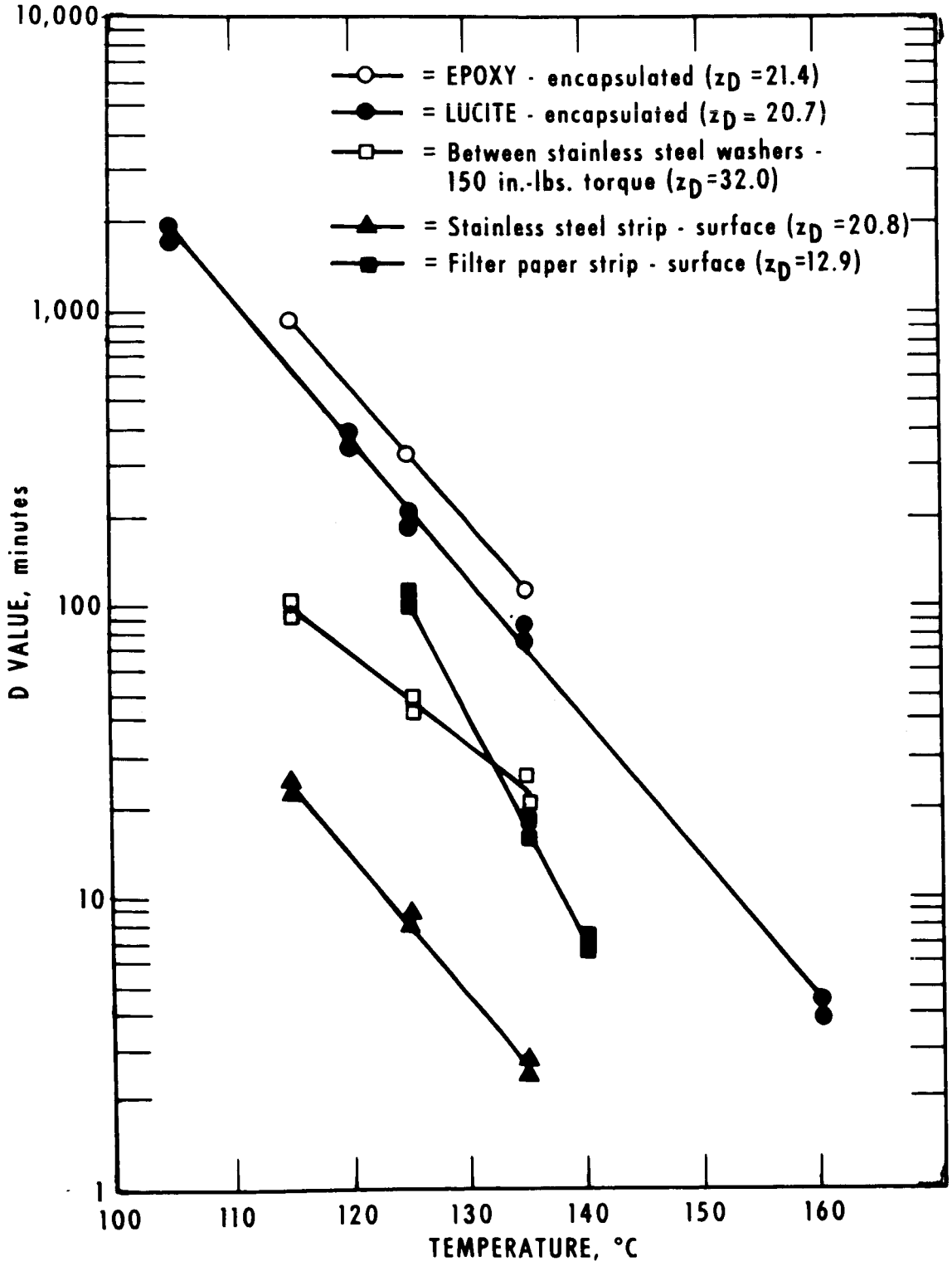

DRY HEAT INACTIVATION AT 115°C OF Bacillus subtilis var. niger SPORES ENCAPSULATED IN EPOXY RESIN

FIGURE 7



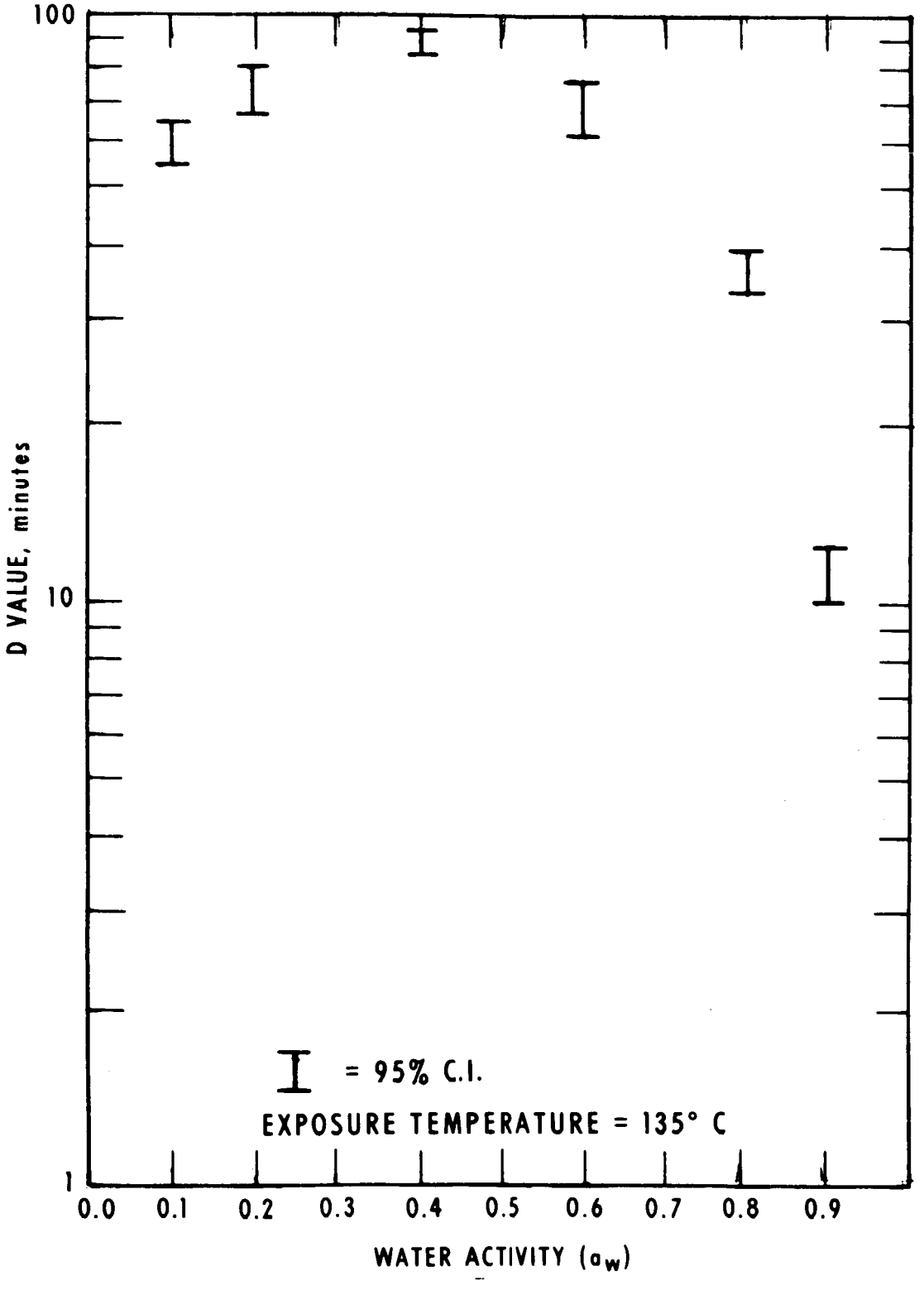

INACTIVATION OF Bacillus subtilis var. niger SPORES IN OR ON VARIOUS MATERIALS EXPOSED TO A DRY HEAT TEMPERATURE OF 135°C.

FIGURE 8




THERMAL DESTRUCTION CURVES
FOR Bacillus subtilis var. niger SPORES

FIGURE 9




INFLUENCE OF WATER ACTIVITY ON DRY HEAT RESISTANCE OF Bacillus subtilis var. niger SPORES ENCAPSULATED IN LUCITE